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# Steered Molecular Dynamics as a Virtual Atomic Force Microscope

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Two examples of non-standard applications of computer modeling of protein dynamics are discussed. External harmonic force attached to selected atoms closely simulates a force exerted by a tip in atomic force microscope in single molecule stretching experiments. Preliminary results of forced unfolding of oncogenic protein gankyrin and forced ligand undocking from photoactive industrial enzyme nitrile hydratase are presented.

## 1 Introduction

Computer modeling based on simple laws of classical mechanics are successfully used in interpretation of single molecule atomic force microscopy experiments<sup>1-4</sup>. The steered molecular dynamics (SMD)<sup>5</sup> or its variants are more and more popular. However, the methodology of doing effective computer experiments is still far from being well established. In this paper preliminary results of applications of the steered molecular dynamics method (SMD) to stretching single proteins are presented. Gankyrin (GAN) is recently discovered oncogenic protein which is highly expressed in many tumors<sup>6</sup>. Its modular structure is close to ankyrins studied earlier<sup>5</sup>, but the molecule has only 7 structurally similar subunits. A small size makes GAN a perfect model system for testing SMD computer models. No experimental AFM data or MD simulations have been published so far.

In nitrile hydratase (NHase), biotechnological enzyme used for production of acrylamide, there are two subunits separated by wide channel leading to the unusual active site containing Fe or Co metal ions<sup>8</sup>. The details how the substrate reaches deeply buried catalytic center nor how the product leaves this cavity are not known. The rational modeling new industrial enzymes, for example, having a better stereoselectivity, requires knowledge on steric determinants of NHase activity. We propose that forced unfolding of ligands, both substrates and products, using virtual AFM is promising strategy for further studies of enzymatic mechanisms.

## 2 Methods

For gankyrin (GAN) simulations the 1TR4 pdb structure was used, for Co-Nhase a 1IRE structure was adopted. NAMD code<sup>7</sup> with CHARM27 force field running on a local linux cluster was used in SMD simulations. To perform Co-Nhase modeling an extensive set of new parameters for non-corrine active site has been developed<sup>8</sup>. Two limiting models of GAN simulations were tested: (a) GAN in a large TIP3 water box and periodic boundary

conditions, 1.45 ns simulation time,  $v = 0.1 \text{ \AA/ps}$ , spring constant  $K = 7 \text{ kcal/mol/\AA}^2$  (b) initial GAN structure surrounded by a droplet of water, 145 ns simulation time,  $v = 0.001 \text{ \AA/ps}$ ,  $K = 14 \text{ kcal/mol/\AA}^2$ . A Langevin coupling to the thermal bath at 300 K was assumed. External forces were attached to CA atom of LEU G8, while GLY G214 CA atom was held fixed. The pulling speed  $v$  seems to be the most critical parameter affecting SMD simulations. We estimated this parameter that the dimension of GAN approximately doubled after a period of time planned for the experiment. Data were analyzed using computer graphics and the VMD code<sup>9</sup>.

### 3 Results and Discussion

#### 3.1 Forced Unfolding of Gankyrin

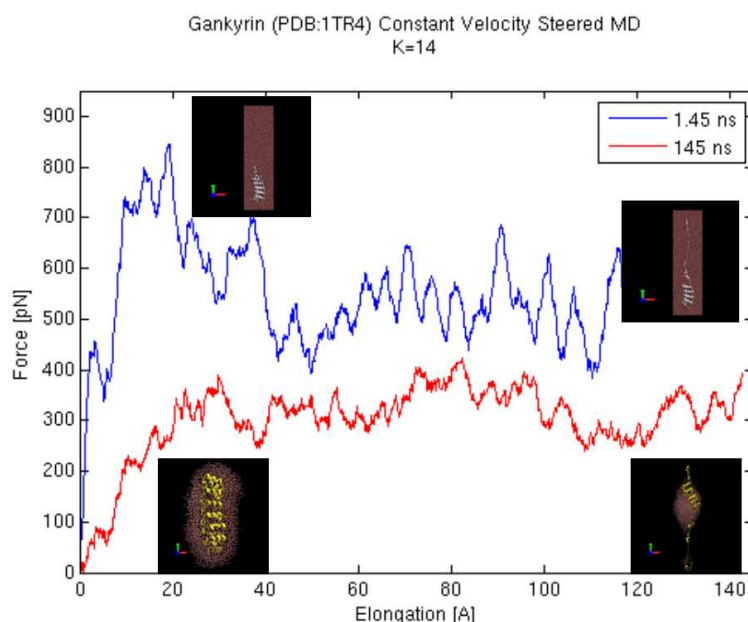


Figure 1. Dependence of force-elongation spectra on SMD pulling velocity in gankyrin.

AFM experiments performed for 24 unit ankyrin<sup>10</sup> have shown that for the initial unfolding of this nanospring a force of the 250 pN is sufficient. Consecutive subunits unfold under even smaller stress of 20-50 pN. Results of our two GAN simulations are shown in Fig. 1. One can immediately see that in 1.45 ns simulation (a) the maximum observed force of 800 pN is far too high. When small pulling speed is applied, in 145 ns simulation a factor of two lower forces are observed. They are still slightly higher than in the related experiment, but this seems to be acceptable. The problem with long simulations is a proper treatment of water, insets in Fig. 1 show that in long simulation a strong lag in water position

is observed. The water shell becomes quite thin in certain regions of the highly stretched GAN, and this may also result in artifacts lowering unfolding forces. Our experiments show that some algorithms weakly bounding water with the protein undergoing constant elongation (or other large conformational change) have to be developed for effective use of SMD approach. PBC with huge water boxes seem to be too expensive and unpractical.

### 3.2 Forced Undocking of Nitrile Hydrates Ligands

It is interesting to check what minimal forces are expected if we pull out the well known ligand from the protein interior. This limiting value is perhaps affected not only of the pulling speed, but also by the nature of ligand-protein interactions and an assumed trajectory of the extracted ligand. In Fig. 2 force-travel distance curves are presented for undocking of a substrate (nicotinonitrile, NCN) and a product (nicotinoamide, NCA) from the best docked position within the NHase interior<sup>8</sup>. A rather slow pulling out is a different process for both molecules, despite their similar sizes. We attribute these differences to H-bonds of NCA with appropriate residues of the NHase channel. Critical positions in space determined in these two computer experiment should be good starting points for systematic mutagenesis studies of Co-NHase.

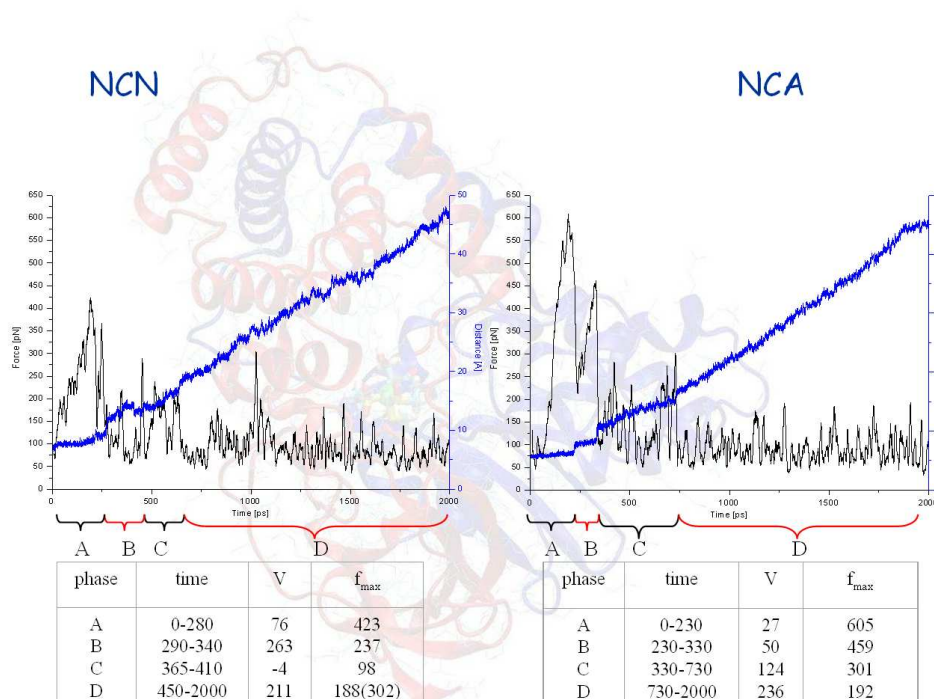


Figure 2. Dependence of SMD force on simulation time and distance traveled by substrate (NCN) and product (NCA) in Co-NHase. Four phases of motion A-D are recognized.

## 4 Conclusions

The model protein GAN unfolding process studied by the SMD method on two time-scales (different by a factor of 100) shows quantitatively distinct features in force-extended curves. Forced undocking of NHase ligand gives useful hints for determining the best opportunities for tailored enzyme construction. New methodology of keeping droplet of a water close to the protein is required before a virtual AFM may be used as a common tool for studying mechanical properties of single biomolecules.

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